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The CEA family: *a system in transitional evolution?*

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ABSTRACT: The CEA family consists of two structurally and functionally distinct sub-groups; the group including CEA, NCA and CGM-6 which are cell surface-bound by phosphatidyl-inositol (PI) linkages, and the group of BGP splice variants which have trans-membrane and cytoplasmic domains. Although all CEA family members mediate intercellular adhesion in vitro, *the PI-linked group show* Ca^{++} *and temperature independent adhesion whereas the BGP group show rapidly reversible* Ca^{++} *and temperature dependent adhesion. From the close alignment in cDNA nucleotide sequences between family members and between repeated domains in one family member, it is apparent that the CEA family is now rapidly evolving; in fact, analogs of only the trans-membrane BGP group have been found so far in the mouse. The addition of a new group of potent adhesion molecules to complex species at some time after the rodent radiation has strong evolutional implications, which are discussed. (Int J Biol Markers, 1992; 7: 137-42)*

KEY WORDS: CEA family, Intercellular adhesion, CEA function

INTRODUCTION

Human carcinoembryonic antigen (CEA) is a member of a family of cell surface glycoproteins which appear in the blood in association with a wide variety of cancers, including colon, breast and lung (1). The cloning of genes and cDNA's for CEA in 1987 (2-8) allowed the delineation of the complete structure of CEA itself and a means for clarification of the extent of the family and the precise structures of all of its members. Considering only the CEA branch of the CEA family (the other branch being the pregnancy-specific glycoproteins), 9 genes have been identified which are closely clustered in two groups on human chromosome 19 (9, 10, 11). Figure 1 depicts the basic structures of the CEA family members for which protein products have been reasonably well characterized. As a subset of the immunoglobulin supergene family, all members consist of a processed leader sequence, followed by a V-like N-terminal domain, a variable number (from 0 to 6) of C2-set domains each with a disulphide bridge, and either a hydrophobic C-terminal domain, which is processed to give a phosphatidyl-inositol (PI) type of linkage to the external cell membrane (CEA, NCA and HCGM-6) (14-17), or a transmembrane domain with a short or a long cytoplasmic tail (splice variants of BGP) (18, 19).

The function(s) of the CEA family was completely unknown prior to the availability of full-length cDNA clones which allowed functional studies in transfectants. CEA (20, 21), NCA (21,22,24) and BGP (23) have all been shown to function *in vitro* as homotypic intercellular adhesion molecules using stable transfectant clones of previously non-adhesive rodent cell lines. HCGM-6 transfectants, on the other hand, appear to interact only heterotypically with NCA transfectants (22, Ilyantis and Stanners, unpublished results). These data together with results showing a lack of heterotypic interactions with transfectants of other adhesion molecules of the immunoglobulin supergene family such as N-CAM and L-MAG (24), indicate that the interactions are specific.

The characteristics of the adhesive process for the phosphatidyl-inositol linked family members (CEA, NCA and HCGM-6) differ from those of the trans-membrane linked family members (BGP) in that the BGP members require calcium and physiological temperatures for adhesion while the CEA group show adhesive function in the absence of calcium and at 4° C (20-23).

In addition to this intercellular adhesive function for CEA family members, the sugars of CEA, especially the mannose-rich sugar structures on one of the glycoforms of NCA, have been shown to bind gram negative bacteria (25). The question of further functions for CEA remains open, especially since other receptors which bind CEA have been demonstrated (26).

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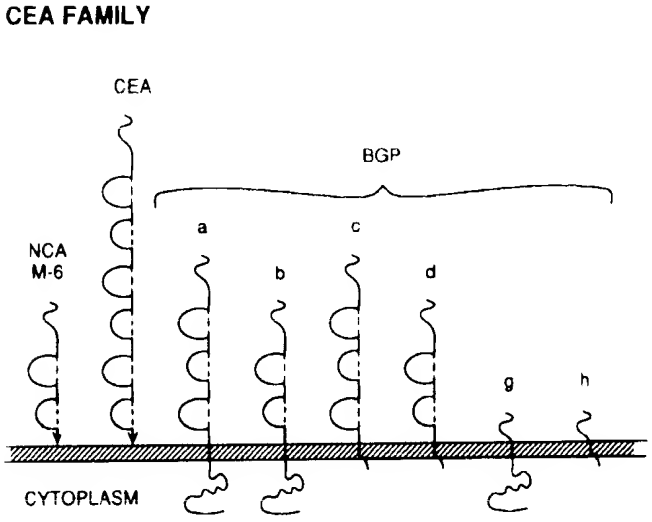

Fig. 1 - *Structure of several members of the CEA family. The V-like Ig N domains are shown as linear termini, while the C2-set domains are shown with loops linked by disulphide bridges. PI-linkage to the external cell membrane is depicted with an arrowhead; transmembrane and cytoplasmic domains are shown for the BGP members, although the configuration with respect to the cell membrane for BGP_g and BGP_h is inferred from the nucleotide-amino acid sequence.*

Notwithstanding this recent progress in delineating *in vitro* functions for CEA, the demonstration that these function(s) apply *in vivo* has yet to be shown. This, of course, represents a very difficult task. If the intercellular adhesion function also applies *in vivo*, one might expect a role in embryonic development and/or in the maintenance of tissue architecture. It is the purpose of this article to use comparative data from both human and animal systems in order to examine the question of function from an evolutionary perspective.

RESULTS

Further delineation of BGP function(s): the calcium and temperature dependence observed for intercellular adhesion mediated by BGPa and BGPb (23) represent important attributes of adhesion which are also characteristic of the cadherin family of adhesion molecules (27), although, recently, calcium-dependent adhesion has been observed for another member of the immunoglobulin supergene family, PECAM-1 (28). This does not imply that the BGP members are necessarily involved in the same types of functions as the cadherins, such as the initiation and maintenance of functional intercellular junctions. It does, however, raise the possibility that the BGP-mediated adhesion could be modulated rapidly by changes in extracellular conditions such as divalent cation concentrations, perhaps by direct "ecto" effects involving the extracellular domains of the molecules. These extracellular events could include direct effects of divalent cations on molecular conformation, ecto-ATPase activity (29), and ecto-phosphorylation of BGP molecules, as suggested by Obrink for the rat analog of human BGP, cell-CAM 105 (30). It is of interest that a cDNA cloned using amino-acid sequence information derived from purified rat ecto-ATPase turned out to be identical to this relatively well characterized adhesion molecule (31). We have looked for ecto-ATPase activity in purified preparations of human BGPa using several assays, without convincingly positive results (Sadoul-Schellenberger and Stanners, unpublished results); it is possible, however, that such activity could depend on integration of BGP molecules in the cell membrane or on associated molecules. We have shown, nevertheless, that BGP adhesion depends on Mg^{++} as well as Ca^{++} (data not shown). This makes it more likely that the effect of these cations is direct, presumably on molecular conformation, i.e., "ecto", rather than indirect through effects on the physiological state of the cell. Work is in progress to study the effect of divalent cations on the molecular conformation of BGP directly.

With regard to reversibility of adhesion, the aggregation of BGP transfectants has been studied as a function of changing extracellular conditions. For these studies, clones of the CHO derived cell line, LR-73 (32), stably transfected with functional cDNA of a splice variant of BGP known as BGPh, were used. This variant contains the least of the possible coding sequences found in BGP splice variants, consisting of the leader, the first 68 amino acids of the N domain coupled directly to the transmembrane domain and the short cytoplasmic domain of 9 amino acids (T. Barnett, personal communication). Adhesion was studied, in comparison with stable LR-73 transfectants of CEA and E-cadherin, as a function of temperature (Fig. 2) and extracellular ATP (Fig. 3). In the first place it is evident that this severely truncated BGP variant is capable of mediating adhesion, unlike CEA whose complete N domain alone is incapable of adhesion (Zhou and Stanners, in preparation). Secondly, the cellular aggregates formed through the action of this molecule rapidly dissociate in response to a temperature shift from 37° C to 4° C, and rapidly reform when the temperature is shifted back to 37° C (Fig. 2). These changes were not observed for transfectants of CEA (Fig. 2) or for NCA transfectants (data not shown). In

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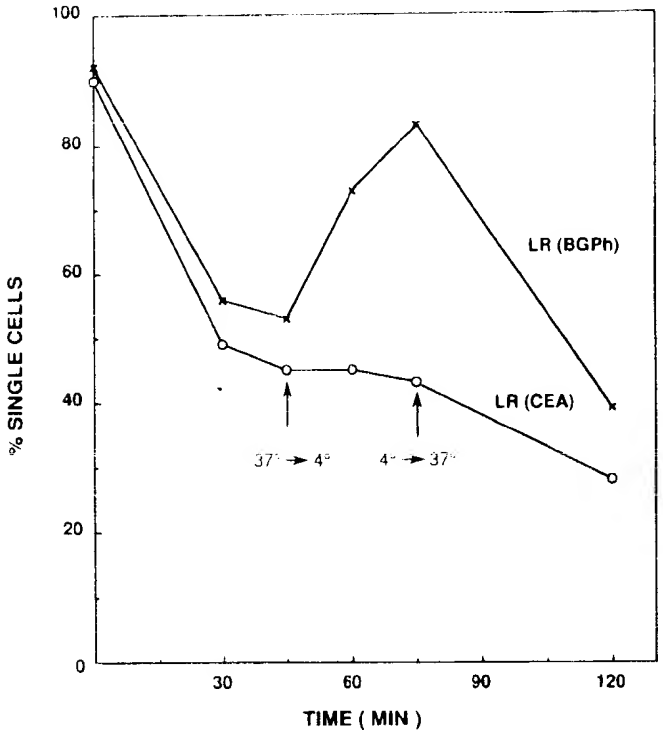

Fig. 2 - *Aggregation of BGP_h and CEA transfectants as a function of rapid shifts in temperature. Clones of LR-73 cells stably transfected with functional CEA or BGP_h cDNA were produced and grown in monolayer culture as previously described (20). After rendering cultures single cell suspensions, the percentage of single cells during the formation and dissociation of aggregates was measured with time as previously described (20). Rapid changes in temperature were effected at the indicated times.*

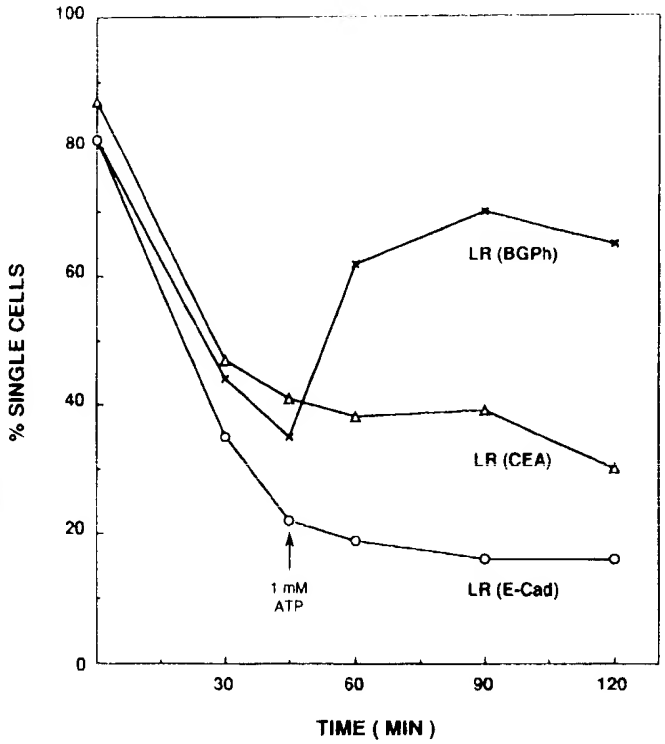

Fig. 3 - *Aggregation of BGP_h, CEA, and E-cadherin transfectants before and after the addition of ATP. The aggregation and dissociation with time of suspended cells from cultures of stable BGP_h, CEA and E-cadherin cDNA transfectant clones in the absence and presence of 1mM ATP were measured as described in the legend of Figure 1.*

addition, aggregates of BGP transfectant cells dissociated immediately after the addition of ATP, whereas CEA and E-cadherin transfectants were unaffected (Fig. 3). This effect was not seen with ADP (data not shown), and could be due to ATP forming complexes and removing Ca^{++} from the medium or could be a direct effect through an ecto-ATPase or ecto-phosphorylation function. Similar rapid reversibility of aggregation was obtained with adjustment of the Ca^{++} concentration in the cellular suspension medium (data not shown).

Extent of CEA family in mice: a restriction fragment of human CEA cDNA from the translational start codon to about the middle of the second C2-set repeated domain was used as a probe to isolate cDNA clones encoding mouse analogs of the human CEA family. A series of clones representing splice variants of what is probably a single gene have been characterized (33, 34). These all have transmembrane domains and either a short or a long cytoplasmic domain of 10 or 73 amino acids, with variable N and C2-set domains. Transfectants of mouse cells with two of these murine splice variants have been shown to demonstrate homotypic intercellular adhesion (33,34) and one has been shown to be temperature and Ca^{++}-dependent (33). The other effected Ca^{++}-independent adhesion, but this could have been due to a very high level of production observed for this particular transfectant clone, an effect also observed for high producing transfectants of human BGP (Rojas and Stanners, in preparation). In addition, the tissue-specific pattern of expression of the murine analogs resembles that of human BGP (30, 33). It should also be pointed out that the amino-acid sequence alignment of the murine family members is closer with human BGP than with human CEA or NCA (33, 34). Thus, all of the murine CEA-like cDNA clones isolated to date resemble human BGP both in structure, function and expression patterns. No transcripts corresponding to PI-linked murine family mem-

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bers analogous to CEA, NCA and HCGM-6 have been discovered to date either in cDNA libraries of murine colon, on Northern blots of RNA from many murine tissues, or by PCR amplification of mouse colon and liver RNA (McCuaig et al, in preparation).

DISCUSSION

The above results add to our previous data showing that the BGP members of the human CEA family differ markedly in the characteristics of their intercellular adhesion function from those of the PI-linked members. These differences include Ca^{++} dependence, temperature dependence and immediate reversibility of adhesion by extracellular conditions; the latter imply "ecto" control of adhesion through rapid changes in molecular conformation. Where tested, these functional properties are shared by the CEA-like molecules which we have detected in the mouse. This leads to the suggestion that the PI-linked members of the human CEA family represent an evolution of a new branch of the family with an evolved adhesive function.

Although it may be presumptuous to suggest that the PI-linked CEA family members do not in fact exist in the mouse, all our evidence to date points in that direction; definitive evidence will require detailed genomic studies and/or careful PCR analysis of mRNA from many tissues of the mouse. If so, we are faced with the prospect of a group of fairly potent intercellular adhesion molecules which have been introduced into the human lineage at some point after the radiation of murine species. This suggestion of a recent evolution of the CEA family is supported by the very close nucleotide sequence alignment ($> 80\%$) between the repeated double C2-set domains in CEA and between CEA, NCA and HCGM-6. Of further interest is the fact that, although there are regions where very little change has occurred, the amino-acid sequence alignment in these comparisons is always less than the nucleotide sequence alignment (4). This is, in fact, exactly as predicted for random changes in the nucleotide sequence without preservation of the actual amino-acid sequence (4). What has been conserved is, as mentioned above, certain limited regions of both the nucleotide and the amino-acid sequence, and the open reading frames; what has evolved are changes in the carboxy terminal sequence to effect PI-linkage. It is possibly the latter linkage that has allowed the evolution of intercellular adhesion molecules with different adhesive characteristics.

The PI-linked CEA family molecules must present some advantage to the organism as they have evolved as such and their open reading frames have been conserved. If they do, in fact, function as intercellular adhesion molecules during embryonic development and in adult tissues, how can mice with their very similar developmental and adult tissue architecture do without them (assuming that the failure to detect them in mice is confirmed)? The answer is, perhaps, a testament to the plasticity of complex biological systems. Since many genes can be "knocked out" in such organisms without apparent phenotypic effect, it is clear that conserved genes with advantageous functions must exist and persist by conferring an inapparent selective advantage. Consistent with this view, the addition of functional CEA nucleotide sequences driven by the SV40 transcriptional promoter to the genome of mice by transgenesis had no apparent effect on their phenotype, even though expression was observed in many tissues (35).

These considerations may provide an explanation for the paradox of the existence of a group of genes in humans but not in mice with a potential function which should affect development. They do not, of course, ratify an intercellular adhesion function for these genes *in vivo*. A highly speculative suggestion is that the PI-linked members of the CEA family represent part of a gene family in "transitional evolution". A simplistic view of molecular evolution would envision the replication of existing genes or domains/exons followed by drift in the amino-acid sequence until a new or modified function capable of conferring selective advantage emerges. All that is required in the transitional period are preservation of the open reading frame and a function which has small advantage and which is at least not deleterious. The CEA family in man could presently be in such a state as viewed through the present narrow window in evolutionary time. The significance of overproduction of the PI-linked family members in tumours is, of course, a completely separate issue.

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